*Brain Research*, 325 (1985) 345–348
Elsevier

BRE 20569



# Development of the rat pineal $\alpha_1$-adrenoceptor

DAVID SUGDEN and DAVID C. KLEIN

*Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205 (U.S.A.)*

(Accepted August 28th, 1984)

*Key words:* pineal — $\alpha_1$-adrenoceptor — development — aging

Pineal $\alpha_1$-adrenoceptors in rats from 19 days of gestation until 11 months of age were studied using [$^{125}$I]iodo-2-[$\beta$-(4-hydroxyphenyl)ethylaminomethyl]tetralone ([$^{125}$I]HEAT). The number of specific [$^{125}$I]HEAT binding sites increased markedly between 18 days of gestation (101.7 ± 13.1 fmol/mg protein) and 10 days of age (336.2 ± 34.3 fmol/mg protein). A significant decline occurred after 1 month of age. A saturation study showed similar changes in receptor density with age ($B_{max}$; 20 days of gestation, 130.5 fmol/mg protein; 35 days old, 288.1 fmol/mg protein) but no difference in $K_d$ (58.4 pM at both −1 and +35 days). The developmental appearance of the pineal $\alpha_1$-adrenoceptor and the decline in its density with age are remarkably similar to changes reported for pineal $\beta$-adrenoceptors.

Until recently the noradrenergic regulation of many aspects of pineal biochemistry was thought to be mediated exclusively by pineal $\beta$-adrenoceptors[11]. However, the recent identification of pineal $\alpha_1$-adrenoceptors[17], and the finding that a pineal $\alpha_1$-adrenoceptor mechanism can potentiate the $\beta$-adrenergic induction of N-acetyltransferase (NAT)[13,19] has prompted a re-evaluation of this concept.

Pineal $\beta$-adrenoceptors have been identified in fetal rats as early as 19 days of gestation[1], prior to the development of the sympathetic neural innervation of the gland[10,12], and the ability of adenosine 3′,5′-phosphate (cyclic AMP) to stimulate NAT activity[1]. Pineal $\beta$-adrenoceptor density increases rapidly between 19 days of gestation and the second week of life[1,3,20]. The density of receptors then falls between 1 and 3 months[9] and remains constant thereafter.

It was of interest to determine the pattern of development of the pineal $\alpha_1$-adrenoceptor in view of its physiological role in controlling the induction of NAT, the rate-limiting enzyme in the synthesis of the pineal hormone, melatonin. We now report that pineal $\alpha_1$-adrenoceptors, identified using ([$^{125}$I]HEAT, are present on day 18 of gestation, and show a rapid increase in number during the first 10 days of life. Thereafter the number of $\alpha_1$-adrenoceptors falls between 1 and 3 months, then remains constant at least until 11 months of age. The similarity in the pattern of development of pineal $\alpha_1$- and $\beta$-adrenoceptors is discussed.

[$^{125}$I]Iodo-2-[$\beta$-(4-hydroxyphenyl)ethylaminomethyl]tetralone ([$^{125}$I]HEAT; 2200 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Unlabeled 2-[$\beta$-(4-hydroxyphenyl)ethylaminomethyl]tetralone HCl (HEAT) was a generous gift of Dr. Hansen, Beiersdorf Ltd. (Hamburg, F.R.G.). The dye binding reagent used for protein determination was purchased from Bio-Rad (Richmond, CA) and all other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Sprague–Dawley rats of known age or conception date, were obtained from Zivic Miller Laboratories (Allison Park, PA). Male rats were used at 1, 3, 6 and 11 months of age and mixed-sex litters at all other ages. All rats were housed in our facilities for at least 3 days before use under a diurnal lighting cycle (L:D 14:10, lights on 05.00 h). Rats were decapitated and pineal glands were removed, when necessary with the aid of a dissecting microscope, between 10.00 and 16.00 h and frozen in microtubes on solid $CO_2$.

*Correspondence:* D. Sugden, Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, Bldg. 10, Rm. 8D42C, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205, U.S.A.

Glands were stored at −40 °C.

Pineals were prepared immediately before use by brief sonication (4 °C). Samples consisted of a single gland at 1, 3, 6 and 11 months of age, 2 glands at 21, 15, 10 and 7 days old, 3 glands at 5 days old, 4 glands at 3 days old, 6 glands at 1 day old, 8 glands at 1 day before birth and 10 to 13 glands at 3 days before birth. The saturation study used a pool of 20 glands from 35-day-old male rats and 60 glands from rats killed 1 day before birth. Binding of [$^{125}$I]HEAT to $\alpha_1$-adrenoceptors was performed essentially as described previously[7,8,17]. Briefly [$^{125}$I]HEAT (200 pM) was incubated with the pineal sample (30–80 $\mu$g of total protein) at 30 °C for 15 min in 250 $\mu$l of Tris-HCl buffer (50 mM, pH 7.4) containing ethylene diamine tetra-acetic acid (EDTA) (1 mM), $Mg^{2+}$ (10 mM) and phenylmethylsulfonyl fluoride (PMSF; 6 $\mu$M). In the saturation study [$^{125}$I]HEAT at a final concentration of 10 to 400 pM was used. Non-specific binding was determined in each sample using HEAT (1 $\mu$M). Free and bound ligand were separated by filtration. The sample protein concentration was determined using a dye-binding method[5] with bovine serum albumin as the standard.

Preliminary studies comparing the characteristics of [$^{125}$I]HEAT binding in a pineal sonicate and membrane (10,000 *g*, 30 min, 4 °C) preparation indicated a single class of non-cooperative binding sites in both preparations. $K_d$ values were similar (sonicate, 57 pM; membrane 41 pM). The majority (> 80%) of pineal [$^{125}$I]HEAT binding sites were recovered in the 10,000 *g* pellet. The pharmacological specificity of the sites identified by [$^{125}$I]HEAT in pineal sonicates was very similar to that in pineal membrane preparations ($K_i$ (sonicate preparation) (nM): prazosin, 0.27; yohimbine, 600; (−)-propranolol, 6000; $K_i$ (membrane preparation) (nM): prazosin, 0.28; yohimbine, 960; (−)-propranolol, 19,000), and was typical of an $\alpha_1$-adrenoceptor[16].

Bartlett's test for heterogeneity of variance[4] followed by Duncan's multiple range test[6] were used to determine the significance of the changes in $\alpha_1$-adrenoceptor density with age. Data from the saturation studies were analyzed by computer using a nonlinear model fitting program[14]. $K_d$ and $B_{max}$ errors indicate the approximate standard errors generated by this program.

Specific [$^{125}$I]HEAT binding was readily detectable in the pineal gland 3 days before birth (Fig. 1). The number of sites increased almost linearly between 3 days before birth and 10 days of age. A small decline in specific [$^{125}$I]HEAT binding sites occurred after this time but the values at 15, 21 and 35 days were not statistically significantly different from the value at 10 days of age.

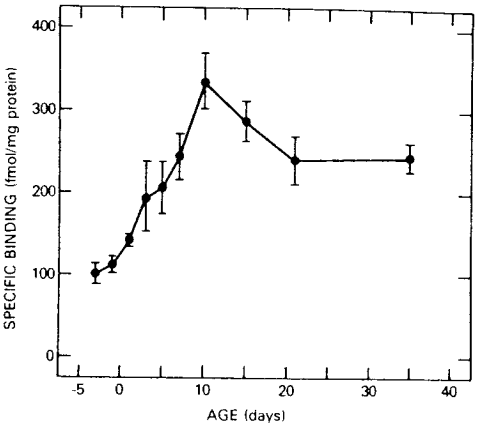


Fig. 1. Developmental appearance of rat pineal $\alpha_1$-adrenoceptors. Pineal glands obtained from rats aged −3 to +35 days were prepared as described in the text. Specific binding of [$^{125}$I]HEAT (defined using HEAT, 1 $\mu$M) was determined for each sample. Three to 7 samples were assayed at each age. Each point represents the mean ± S.E.M.

Scatchard analysis of data obtained using a pool of pineal glands taken from rats 1 day before birth or at 35 days of age revealed a single class of non-cooperative binding sites (Fig. 2). $K_d$ values at both ages

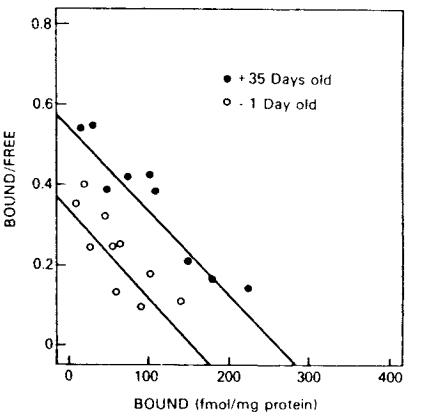


Fig. 2. Scatchard plot of [$^{125}$I]HEAT binding in pineals taken from rats aged −1 or +35 days old. Triplicate samples (~40 $\mu$ of protein) were incubated (30 °C; 15 min) at each concentration of [$^{125}$I]HEAT. Specifically bound [$^{125}$I]HEAT was plotted against the bound/free ratio. The best straight line was fitted to the data using non-weighted linear regression.

were identical (−1 day, 58.4 ± 39.9 pM; +35 days, 58.4 ± 28.5 pM) and not substantially different from values previously reported for adult pineal membranes[17]. $B_{max}$ values were similar to those obtained using a single, saturating concentration of [$^{125}$I]HEAT (−1 day, 130.5 ± 58.5 fmol/mg protein; +35 days, 288.1 ± 93.0 fmol/mg protein).

A statistically significant fall in the number of specific [$^{125}$I]HEAT binding sites occurred between 1 and 3 months of age (Fig. 3). Values for 3, 6 and 11 months were not significantly different.

Pineal $\alpha_1$-adrenoceptors, identified using the specific, high-affinity $\alpha_1$-adrenoceptor ligand, [$^{125}$I]HEAT, were present 3 days before birth (Fig. 1), before any significant innervation of the gland by the sympathetic nervous system is apparent[10,12]. A rapid increase in the number of $\alpha_1$-adrenoceptors was seen between −3 and 10 days of age; a period during which neuronal development in the gland is intense. Interestingly, in the adult rat, pineal $\alpha_1$-adrenoceptor density has been shown to be under neural control, since interruption of neuronal stimulation by superior cervical ganglionectomy increased

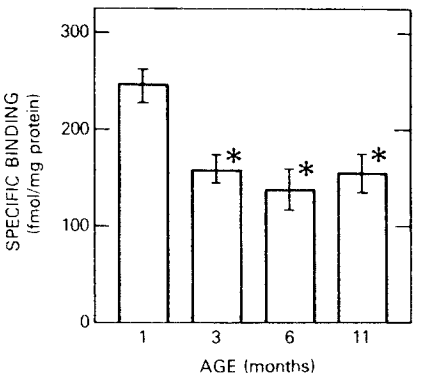


Fig. 3. Changes in specific [$^{125}$I]HEAT binding in pineals from aging rats. Specific binding of [$^{125}$I]HEAT was determined as described in the test. Each point represents the mean ± S.E.M. specific binding (fmol/mg protein) in 4–7 individual pineals. * $P < 0.05$ compared to 1-month-old rats.

receptor number[18]. Perhaps the slowing of the rise in $\alpha_1$-adrenoceptor density after 10 days of age reflects the functional maturation of the sympathetic innervation.

The possibility that neonatal pinealocytes have an undifferentiated adrenergic receptor was raised in a study which demonstrated both $\alpha$- and $\beta$-adrenoceptor-mediated increases in N-acetyltransferase activity in neonatal pineal cells maintained in monolayer culture[15]. Our finding that specific [$^{125}$I]HEAT binding sites are present in pineal membranes taken from neonatal rats and that these sites have an identical affinity for the ligand as in adult pineal membranes (Fig. 2) indicates that $\alpha_1$-adrenoceptors are differentiated before birth and refutes this suggestion. Rather the presence of $\alpha_1$-adrenoceptors in the neonatal rat pineal strengthens our suggestion[13] that the biphasic dose/response curve for cyclic AMP accumulation in neonatal cells treated with isoproterenol[2] reflects an action at $\beta$-adrenoceptors at low concentrations and a synergistic action on $\alpha_1$- and $\beta$-adrenoceptors at higher concentrations, as is observed with norepinephrine in adult pineal glands[13,19].

There are striking similarities in the pattern of development of pineal $\alpha_1$- and $\beta$-adrenoceptors. Both are present before birth and increase rapidly during the first two weeks of life[1,3,20]. In addition, pineal $\alpha_1$-adrenoceptor density decreased between 1 and 3 months of age and remained constant at least until 11 months (Fig. 3). A fall in rat pineal $\beta$-adrenoceptor density at precisely this time has been observed[9]. In addition, $\beta$-adrenoceptor density then remains constant at least until 24 months of age[9]. The similarity of the developmental changes in the density of $\alpha_1$- and $\beta$-adrenoceptors suggests that in pinealocytes the expression of the genes for these two proteins may be controlled by similar mechanisms. The pineal offers an attractive model system for studying the mechanisms which regulate the development of these receptors.

1 Auerbach, D. A., $\beta$-Adrenergic receptors during development. In D. C. Klein (Ed.), *Melatonin Rhythm Generating System: Developmental Aspects,* Karger Press, Basel, 1982, pp. 97–107.

2 Auerbach, D. A., Klein, D. C., Woodard, C. and Aurbach, G. D., Neonatal rat pinealocytes: typical and atypical characteristics of [$^{125}$I]iodohydroxybenzylpindolol binding and adenosine 3′,5′-monophosphate accumulation, *Endocrinology,* 108 (1981) 559–567.

3 Auerbach, D. A., Weller, J. L. and Klein, D. C., Differential rates of development of components of the adrenergic-adenylate cyclase system, *Pediat. Res.,* 14 (1980) 453.

4 Bennett, C. A. and Franklin, M. L., *Statistical Analysis in Chemistry and the Chemical Industry,* Wiley, New York, 1954, 196 pp.
5 Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analyt. Biochem.,* 72 (1976) 248–254.
6 Duncan, D. B., Multiple range and multiple F-tests, *Biometrics,* 11 (1955) 1–42.
7 Glossmann, H. and Lubbecke, F., [$^{125}$I]HEAT: fifty percent of the ligand can bind to the $\alpha_1$-adrenoceptors with extremely high affinity, *Naunyn Schmiedeberg's Arch. Pharmacol.,* 321 (1982) 7–10.
8 Glossmann, H., Lubbecke, F. and Bellemann, P., [$^{125}$I]HEAT, a selective high affinity, high specific activity ligand for $\alpha_1$-adrenoceptors, *Naunyn Schmiedeberg's Arch. Pharmacol.,* 318 (1981) 1–9.
9 Greenberg, L. H. and Weiss, B., $\beta$-Adrenergic receptors in aged rat brain: reduced number and capacity of pineal gland to develop supersensitivity, *Science,* 201 (1978) 61–63.
10 Hakanson, R., Lombard Des Gouttes, M. N. and Owman, C., Activities of tryptophan hydroxylase, DOPA decarboxylase and monoamine oxidase as correlated with the appearance of monoamines in developing rat pineal gland, *Life Sci.,* 6 (1967) 2577–2585.
11 Klein, D. C., Auerbach, D. A., Namboodiri, M. A. A. and Wheler, G. H. T., Indole metabolism in the mammalian pineal gland. In R. J. Reiter (Ed.), *The Pineal Gland: Anatomy and Biochemistry, Vol. 1,* CRC Press, Boca Raton, 1981, pp. 199–227.
12 Klein, D. C., Namboodiri, M. A. A. and Auerbach, D. A., The melatonin generating system: developmental aspects, *Life Sci.,* 28 (1981) 1975–1986.
13 Klein, D. C., Sugden, D. and Weller, J. L., Postsynaptic $\alpha$-adrenergic receptors potentiate the $\beta$-adrenergic stimulation of pineal N-acetyltransferase, *Proc. nat. Acad. Sci. U.S.A.,* 80 (1983) 599–603.
14 Munson, P. J. and Rodbard, D., LIGAND: a versatile computerized approach to characterization of ligand binding systems, *Analyt. Biochem.,* 107 (1980) 220–239.
15 Rowe, V. and Parr, J., Developmental changes in the stimulation of serotonin N-acetyltransferase activity and melatonin synthesis in the rat pineal in monolayer culture, *J. Pharmacol. exp. Ther.,* 218 (1981) 97–102.
16 Starke, K., Alpha-adrenoceptors subclassification, *Rev. Physiol. Biochem. Pharmacol.,* 88 (1981) 199–236.
17 Sugden, D. and Klein, D. C., Rat pineal $\alpha_1$-adrenoceptors: identification and characterization using [$^{125}$I]iodo-2-[$\beta$-(4-hydroxyphenyl)-ethylaminomethyl]tetralone ([$^{125}$I]HEAT), *Endocrinology,* 114 (1984) 435–440.
18 Sugden, D. and Klein, D. C., Regulation of rat pineal $\alpha_1$-adrenoceptors, *J. Neurochem.,* in press.
19 Sugden, D., Weller, J. L., Klein, D. C., Kirk, K. L. and Creveling, C. R., Alpha-adrenergic potentiation of beta-adrenergic stimulation of rat pineal N-acetyltransferase: studies using cirazoline and fluorine analogs of norepinephrine, *Biochem. Pharmacol.,* in press.
20 Weiss, B., Greenberg, L. and Cantor, E., Age-related alterations in the development of adrenergic denervation supersensitivity, *Fed. Proc.,* 38 (1979) 1915–1921.